NEUROSECRETION. X. A RELATIONSHIP BETWEEN THE PARAPHYSIS AND THE PARAVENTRICULAR NUCLEUS IN THE GARTER SNAKE (THAMNOPHIS SP.)[1]

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In an earlier paper in this journal (Scharrer and Scharrer, 1944) it was shown that in vertebrates the stainable material produced by the neurosecretory cells of the hypothalamus passes along the axons of these cells toward the hypophysis. Similar material elaborated by the neurosecretory cells of the pars intercerebralis in insects also "migrates" along nerve fibers toward an organ complex, namely the corpora cardiaca and allata, which corresponds in many ways to the hypophysis of the vertebrates. The concept of two analogous neuroendocrine systems in invertebrates and vertebrates, which was established on the basis of these observations, has proved fruitful. As to the intercerebralis-cardiacum-allatum system of the insects, Thomsen (1948) studied a possible relationship between the neurosecretory activity of the pars intercerebralis and the "gonadotropic" principle of the corpus allatum. The study showed a positive relationship in that extirpation of the pars intercerebralis in the fly, *Calliphora erythrocephala*, prevented the production or release of the gonad-stimulating principle of the corpus allatum and resulted in the inability of the ovary to produce mature eggs. Also, the concept of an analogous hypothalamo-hypophyseal system in vertebrates has been extended further by Bargmann (1949), Bargmann and Hild (1949), Hild (1950), Ortmann (1950), Bargmann, Hild, Ortmann and Schiebler (1950) and Smith (1951). There is now convincing evidence, recently reviewed by Bargmann and Scharrer (1951), that the stainable material produced by the secreting nerve cells of the hypothalamus and passing along their axons to the neurohypophysis is related to the hormones which can be extracted from the pars nervosa of the pituitary gland.

If this conclusion is correct, neither the corpus cardiacum of the insects nor the neurohypophysis of the vertebrates are endocrine organs in a strict sense, but serve principally as sites of storage of the products of neurosecretory cells in the brain.

Questions arise as to whether (a) the neurohypophysis and the corpus cardiacum are the only organs into which the secreting cells of the hypothalamus and the pars intercerebralis respectively discharge their products, and (b) neurosecretory centers other than those in the hypothalamus of the vertebrates and the pars intercerebralis of the insects are also connected with reservoirs which receive the products of neurosecretory activity by way of axons and store them for release according to need. Both problems are at present under investigation. Observations reported in this paper concern a relationship between the neurosecretory cells of the paraventricular nucleus and an organ other than the pars nervosa of the pituitary, namely the paraphysis, in the garter snake.

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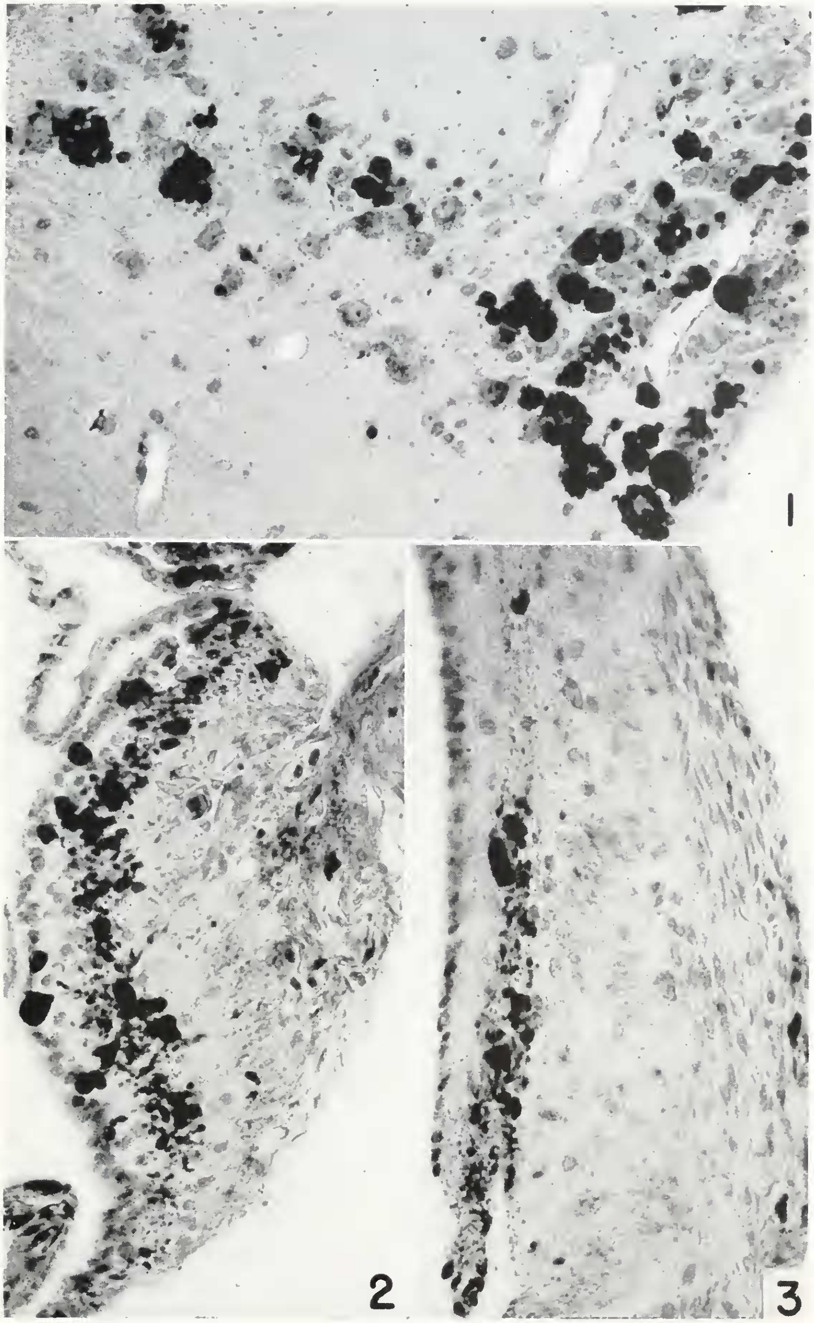

FIGURES 1 to 7: Thamnophis sp., 5 micra sections; Gomori's chrome alum hematoxylin phloxine; photomicrographs.

FIGURE 1. Nucleus supraopticus. Secretory granules and droplets are stained deep blue. × 440.

FIGURE 2. Cross section of stalk of pituitary with stainable material from neurosecretory cells in the hypothalamus. × 400.

FIGURE 3. Longitudinal section through stalk of pituitary with stainable material from neurosecretory cells in the hypothalamus. × 400.

Materials and Methods

The brains of garter snakes (Thamnophis sp.) were fixed with Zenker-formol by immersion or perfusion through the heart. They were embedded in paraffin; sections of 5 micra thickness were stained with chrome alum hematoxylin phloxine as described by Gomori (1941).

Observations

In snakes, as in higher vertebrates, there are two nuclei in the hypothalamus whose cells show secretory activity: the nucleus supraopticus and the nucleus paraventricularis (Scharrer, 1933). The location of these nuclei in the garter snake is illustrated in Figure 8.

The cells of the supraoptic and paraventricular nuclei are distinct in that they contain many fine granules and droplets of varying size. This material stains dark blue with Gomori's chrome alum hematoxylin phloxine method (Fig. 1). The elaboration of this stainable material in granular form is characteristic of the secretory activity of these cells, not only in the snakes but in the homologous nuclei of lower as well as higher vertebrates.

These granules, as in the case of other animals, are contained in the processes of the cells which can, therefore, be traced with great accuracy. It was shown in an earlier paper of this series (Palay, 1945) that in fishes the axons provide a means by which the granules are removed from the neurosecretory cells. This "neurosecretory pathway" in snakes, as in other vertebrates, leads to the pars nervosa of the pituitary gland (Bargmann, Hild, Ortmann and Schiebler, 1950). That an actual transport takes place by a proximo-distal current of axoplasm was suggested by Drager (1950). He replaced the pituitary gland by fibrin foam in the tropical indigo snake (*Spilotes corais*) and found two to three weeks after the operation in the non-living implant granules of a stainable material similar to that elaborated by the neurosecretory cells. Histological observations in the garter snake indicate that the mechanism is the same as in the tropical indigo snake. The pituitary stalk of the garter snake contains large amounts of the stainable material (Figs. 2, 3) which is also found in the neurosecretory cells of the supraoptic and paraventricular nuclei. This material eventually accumulates in the pars nervosa of the hypophysis.

A small number of fibers which arise from cells in the anterior portion of the nucleus paraventricularis and run anteriorly and dorsally toward the commissura pallii posterior (Kappers, Huber and Crosby, 1936, p. 1347) and the paraphysis (Figs. 4, 5), constitute another, hitherto unknown, connection along which neurosecretory granules may be traced. The stainable material accumulates in the commissura pallii posterior in much the same way as in the pituitary stalk (Figs. 6, 7); the amounts of the material vary from individual to individual (Figs. 5, 6). Fibers from the commissura pallii posterior carrying small blue-staining granules can be traced into the paraphysis. No accumulation of the stainable substance seems to take place in the paraphysis; here the granules appear to fade out.

Although the quantities of stainable material involved in the pathway from the paraventricular nucleus to the paraphysis are small by comparison with those seen in the hypothalamo-hypophyseal system, a similar principle seems to obtain in both cases. This similarity of the dorsal and ventral outlets for the secretory products of

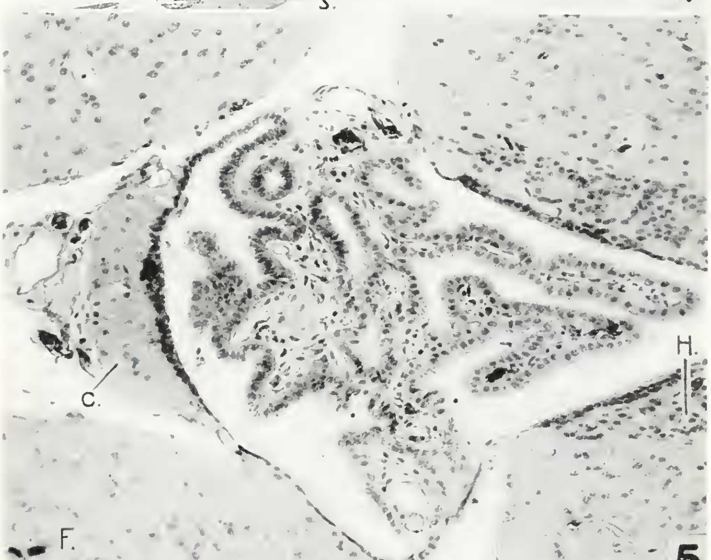

FIGURE 4. Sagittal section through diencephalon. × 40. C., Commissura pallii posterior; E., Epiphysis; M., Midbrain; O., Optic chiasm; P., Paraphysis; PN., Paraventricular nucleus; S., Stalk of pituitary.

FIGURE 5. Basiparallel section through the epithalamus. Note the small amount of stainable material in the commissura pallii posterior as compared with the large amount in the animal shown in Figures 6 and 7. × 180. C., Commissura pallii posterior; F., Forebrain; H., Habenular ganglion.

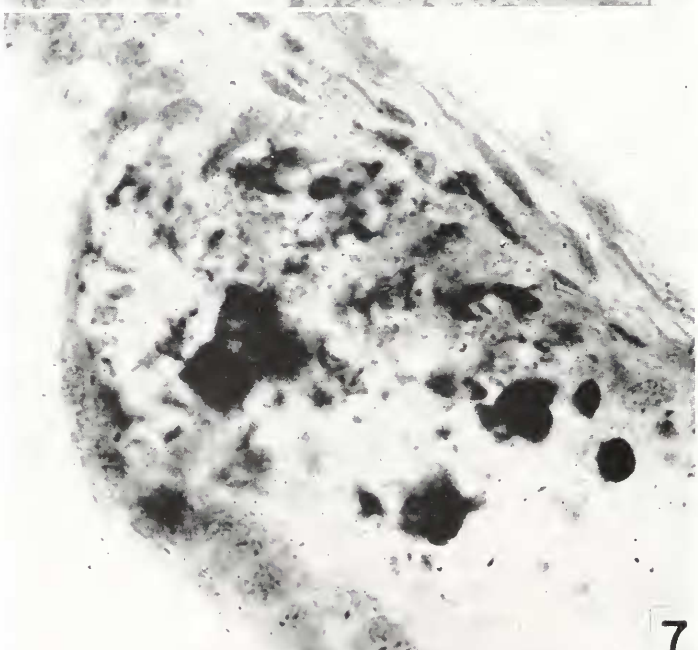

FIGURE 6. Sagittal section through the epithalamus. ×180. C., Commissura pallii posterior; P., Paraphysis.

FIGURE 7. Accumulation of stainable material in the Commissura pallii posterior in the same animal shown in Figures 4 and 6. ×1000.

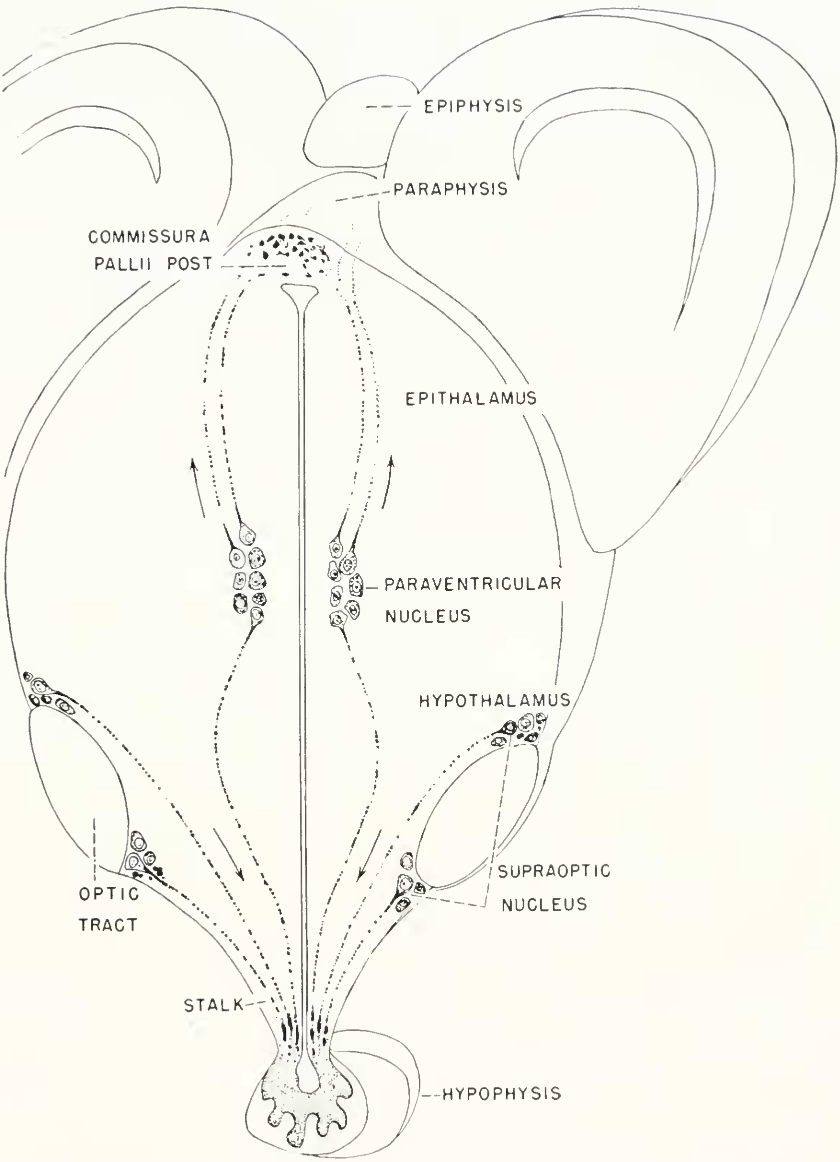

FIGURE 8. Diagram of cross section through the diencephalon of Thamnophis sp. The irregularly spaced dots represent granules of stainable material which mark the axons of neurosecretory cells of the paraventricular and supraoptic nuclei. These granules pass from the neurosecretory cells by way of the axons to (a) the neurohypophysis and (b) the commissura pallii posterior and the paraphysis. The arrows indicate the direction of flow.

the hypothalamus is diagrammatically illustrated in Figure 8. In both cases fine granules of the stainable material "migrate" along the processes of the neurosecretory cells, the larger part going in the direction of the pituitary, a smaller part in the direction of the paraphysis. In the pituitary stalk and in the commissura pallii posterior, the granules coalesce to form large masses of stainable material. In the pars nervosa of the pituitary and in the paraphysis, again we find delicate nerve fibers with the characteristic beadlike arrangement of small blue-staining granules.

Discussion

Tilney (1938) enumerates 10 cerebral "glands," namely (1) the paraphysis, (2) the periphysis, (3) the epiphysis, (4) the mesophysis, (5) the metaphysis, (6) the lateral chorioideal glands, (7) the mesial chorioideal gland, (8) the caudal chorioideal gland, (9) the hypophysis, and (10) the saccus vasculosus. Tilney's outline is useful in that it calls attention to the fact that the pituitary is not the only, but one of several glands which are connected with the brain. His summary may serve as a starting point for the exploration of this interesting and rather obscure field, but after some study it appears that the list of "glands" was drawn up rather schematically and requires revision in several respects. For instance, a large structure overlying the medulla oblongata in the ganoid fishes, which Tilney calls the metaphysis, is not a gland at all, but myeloid tissue (Scharrer, 1944). Similarly, the inclusion of the saccus vasculosus of the teleost fishes among the "glands of the brain" seems questionable. The occurrence of the saccus vasculosus in deep sea fishes, together with its finer structure, innervation, and vascularization suggest that this organ may be concerned with the perception of pressure differences and the maintenance of intracranial pressure equilibrium in vertical movements (Dammermann, 1910; Scharrer, 1948).

Even the criteria which are used in classifying the neurohypophysis as a "gland of the brain" are now under scrutiny. As was pointed out above there is evidence that the stainable material and the extractable hormones of the neurohypophysis are produced by secreting nerve cells in the diencephalon. According to this view the pars nervosa of the pituitary gland is a site of storage rather than of production of hormones and of microscopically visible material.

A relationship between the hypothalamus and the paraphysis is here suggested which so far has been observed only in the genus of snakes used in this study, but which deserves further exploration in other groups of animals. In the garter snake this relationship looks like a mirror image of that which obtains between the hypothalamus and the hypophysis (Fig. 8). It is at present not clear whether the substances produced by the neurosecretory centers of the diencephalon are stored in their active form in the pars nervosa of the pituitary and the commissura pallii posterior, or whether they represent only antecedents necessary for the synthesis of hormones. It also remains undecided at present whether these structures, the pars nervosa and the commissura pallii posterior, together with the paraphysis, serve no other purpose than to store and release the substances which they receive from the neurosecretory cells or whether they themselves produce additional material.

Summary

In snakes, as in other vertebrates, the neurosecretory cells of the supraoptic and paraventricular nuclei discharge their products by way of their axons into the pars

nervosa of the pituitary gland. In the garter snake (Thamnophis sp.) another, hitherto undescribed neurosecretory pathway exists: secretory granules can be traced along processes of cells of the paraventricular nuclei to the commissura pallii posterior and the paraphysis. The granules accumulate in the commissura pallii posterior in a manner similar to that observed in the stalk of the pituitary.

LITERATURE CITED

Bargmann, W., 1949. Über die neurosekretorische Verknüpfung von Hypothalamus und Hypophyse. *Klin. Wschr.*, **27**: 617–622.

Bargmann, W., and W. Hild, 1949. Über die Morphologie der neurosekretorischen Verknüpfung von Hypothalamus und Neurohypophyse. *Acta Anat.*, **8**: 264–280.

Bargmann, W., W. Hild, R. Ortmann and Th. H. Schiebler, 1950. Morphologische und experimentelle Untersuchungen über das hypothalamisch-hypophysäre System. *Acta neuroveg.*, **1**: 233–275.

Bargmann, W., and E. Scharrer, 1951. The site of origin of the hormones of the posterior pituitary. *Amer. Scientist*, **39**: 255–259.

Dammermann, K. W., 1910. Der Saccus vasculosus der Fische ein Tiefeorgan. *Zeitschr. f. wiss. Zool.*, **96**: 654–726.

Drager, G. A., 1950. Neurosecretion following hypophysectomy. *Proc. Soc. Exp. Biol. Med.*, **75**: 712–713.

Gomori, G., 1941. Observations with differential stains on human islets of Langerhans. *Am. Jour. Pathol.*, **17**: 395–406.

Hild, W., 1950. Zur Frage der Neurosekretion im Zwischenhirn der Schleie (*Tinca vulgaris*) und ihrer Beziehungen zur Neurohypophyse. *Zeitschr. f. Zellforsch.*, **35**: 33–46.

Kappers, C. U. A., G. C. Huber and E. C. Crosby, 1936. The comparative anatomy of the nervous system of vertebrates, including man. 2 vols. Macmillan Co., New York.

Ortmann, R., 1950. Morphologisch-experimentelle Untersuchungen über das diencephal-hypophysäre System im Verhältnis zum Wasserhaushalt. *Klin. Wschr.*, **28**: 449.

Palay, S. L., 1945. Neurosecretion VII. The preoptico-hypophysial pathway in fishes. *J. Comp. Neur.*, **82**: 129–143.

Scharrer, E., 1933. Die Erklärung der scheinbar pathologischen Zellbilder im Nucleus supraopticus und Nucleus paraventricularis. *Zeitschr. ges. Neur. Psychiat.*, **145**: 462–470.

Scharrer, E., 1944. The histology of the meningeal myeloid tissue in the ganoids Amia and Lepisosteus. *Anat. Rec.*, **88**: 291–310.

Scharrer, E., 1948. The blood vessels of the saccus vasculosus. *Anat. Rec.*, **100**: 756.

Scharrer, E., and B. Scharrer, 1944. Neurosecretion VI. A comparison between the intercerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. *Biol. Bull.*, **87**: 242–251.

Smith, S. W., 1951. The correspondence between hypothalamic neurosecretory material and neurohypophysial material in vertebrates. *Amer. J. Anat.* (In press.)

Thomsen, E., 1948. Effect of removal of neurosecretory cells in the brain of adult *Calliphora erythrocephala* Meig. *Nature*, **161**: 439.

Tilney, F., 1938. The glands of the brain with especial reference to the pituitary gland. *Res. Publ. Ass. Nerv. Ment. Dis.*, **17**: 3–47.